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January 1, 1974 - June 30, 1974

A STUDY OF PSYCHROPHILIC ORGANISMS ISOLATED FROM THE MANUFACTURE AND ASSEMBLY AREAS OF SPACECRAFT TO BE USED IN THE VIKING MISSION

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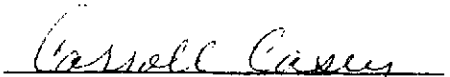

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A STUDY OF PSYCHROPHILIC ORGANISMS ISOLATED FROM THE MANUFACTURE AND ASSEMBLY AREAS OF SPACECRAFT TO BE USED IN THE VIKING MISSION

Portions of this report
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by

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FOREWORD

This fourth semiannual progress report summarizes work performed for the National Aeronautics and Space Administration by the Department of Biology at Hardin-Simmons University, supported by NASA Grant NGR 44-095-001 (Supplement No. 1), and covers the period January 1, 1974 - June 30, 1974.

This report includes investigations of hardy organisms and teflon ribbons from Cape Canaveral performed at the request of the Planetary Quarantine Office. These investigations include the effect of storage of dry-heat-treated teflon ribbons under nitrogen gas followed by high vacuum on the recovery of hardy organisms from the ribbons. A similar experiment was performed on spore crops of hardy organisms recovered previously from Cape Canaveral.

Previous reports have described the use of a slide-culture technique for rapid demonstration of growth in artificial environments. This technique has recently been improved with the acquisition of new microscope equipment, and preliminary results are presented here. In addition, hardy organisms have been inoculated onto slides and subjected to an artificial Martian environment in an attempt to demonstrate their growth in this environment.

Additional experiments using the artificial Martian environment include response of soil samples from the VAB with both constant-temperature and freeze-thaw cycles. These experiments were performed with dried soil and soil containing added water.

Other investigations included in this report are the effect of heat-shock on soil samples (wet and dry), psychrophilic counts of new soil

samples from the manufacture area of the Viking spacecraft, effect of pour plate versus spread plate on psychrophilic counts, and preparation of spore crops of hardy organisms from Cape Canaveral.

The NASA Technical Officer for this grant is Lawrence B. Hall, NASA Planetary Programs, Code SL, Washington, D.C.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

HARDY ORGANISMS AND TEFLON RIBBONS

A. Effect of Storage of Teflon Ribbons Under Dry Nitrogen Followed by Vacuum on the Recovery of Hardy Organisms

Teflon ribbons which had been exposed to the dry-heat cycle proposed for the Viking lander were shipped by air from Cape Canaveral to Abilene for investigation. The ribbons used are presented in Table 1.

Table 1. Teflon ribbons shipped from Cape Canaveral to Abilene for nitrogen-vacuum studies

Date Mailed From Cape Canaveral	Date Received at H-SU	Cape Canaveral Run No.
3-29 Fri.	4-1 Mon.	30
4-1 Mon.	4-4 Thurs.	31
4-5 Fri.	4-8 Mon.	32
4-8 Mon.	4-10 Wed.	33
4-15 Mon.	4-17 Wed.	34

Although preliminary shipments of small numbers of ribbons from Cape Canaveral to JPL and return showed no evidence for contamination, shipment of large numbers of ribbons to our lab showed contamination due to compression and distortion of foam vents during air shipment. This gross contamination resulted in data which is not valid. During recent conversations, new packaging procedures and new ways of handling samples after their arrival at H-SU were discussed, and additional teflon ribbons will be shipped immediately for continued investigation.

B. Response of Spore Crops of Hardy Organisms to Storage in Nitrogen Gas Followed by Vacuum

Procedure. A fresh set of hardy organisms was received from Cape Canaveral and attempts were made to produce spore crops of these (to be discussed in another part of this report). At the time that the teflon ribbons were set up under nitrogen, only four spore crops had been successfully prepared with high titers. One-tenth ml. amounts of titered spore crops of isolates M2-18, M6-25,V2-20,and V5-8 were deposited into duplicate glass vials containing one gram of sterile, dry VAB soil. These were left open under laminar flow to allow alcohol to evaporate, loosely sealed, and placed into a pressure-vacuum container separate from the teflon ribbons. They were then exposed to nitrogen gas followed by vacuum as described for the teflon ribbons. At the end of the nitrogen-vacuum treatment, the samples were diluted in 1% peptone, plated with enriched TSA, and incubated at 32°C to determine changes in population imposed by this treatment.

Results.

Table 2. Population changes of spore suspensions of hardy organisms after storage under nitrogen gas followed by vacuum

Spore Crop	N_o[a] (CFU/g of soil)	Final Population (CFU/g of soil)
M2-18	1.2×10^6	8.1×10^5
M6-25	1.8×10^6	6.7×10^5
V2-20	3.0×10^6	2.9×10^6
V5-8	8.0×10^5	3.6×10^5

[a]Original number of spores deposited in soil

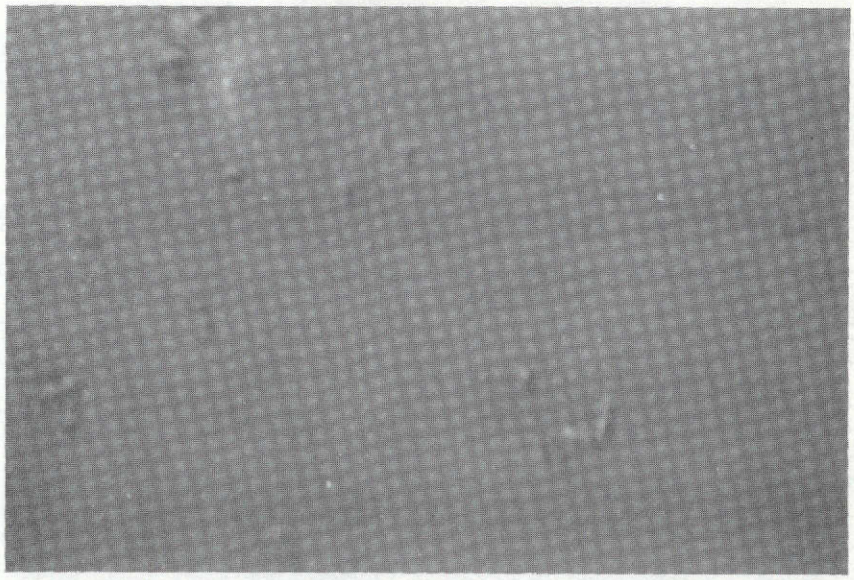
Table 2 indicates that subjecting spores of hardy organisms from Cape Canaveral to a pressure of 5 inches of nitrogen gas for four weeks followed by a vacuum of 10^{-6}Torr for four weeks has no appreciable effect on the crops.

C. Response of Hardy Organisms to Artificial Martian Environment

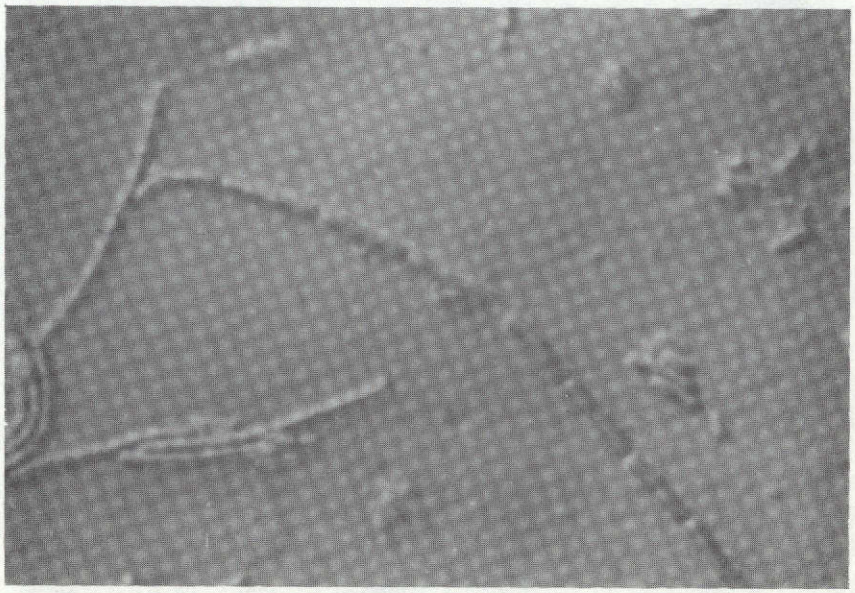
Procedure. Our lab has recently received additional microscope equipment to allow us to perform more observable experiments using the slide culture technique described in Reports No. 2 and 3 (July, 1973 and February, 1974). These new procedures are just now being evaluated, and preliminary results are given on page 22 of this report. Among the first experiments performed with this procedure is an attempt to demonstrate rapid detection of growth of hardy organisms in our artificial Martian environment (described on page 10 in this report).

Briefly, the procedure is to apply spore crops of hardy organisms onto different chambers of an 8-chambered slide which is presently used (VWR #62407-007). Each spore crop is placed onto a non-nutrient agar and TS agar, the slide is placed into Virtis Bulk Vac Bottles, and the bottle is then sealed under the artificial Martian environment and subjected to the freeze-thaw cycle. During this run, samples were incubated in the freeze-thaw cycle for three days, removed, and examined by interference-contrast microscopy to detect the formation of microcolonies as evidence of growth.

Results. Figure 1 demonstrates the response of spore suspensions of hardy organisms to incubation in the experimental Martian environment after 72 hours. As can be seen, there is very good evidence of



(A)



(B)

Fig. 1. Nomarski interference contrast micrographs (1500X) of spore suspensions of hardy organisms in the experimental Martian environment on dry non-nutrient agar (A) and on dry TSA (B) after 72 hours

growth. Figure 1A is the spore suspension on non-nutrient agar after 72 hours, and 1B is the same suspension on TSA after 72 hours, both in our artificial Martian environment. Figure 1A shows the very few of the spores have germinated, and none have grown; whereas Figure 1B shows germination and obvious growth.

As mentioned, these are early results and all spore suspensions of hardy organisms will be run through these same procedures. These early results indicate that the hardy organisms do grow in the artificial Martian environment on TSA which has been dried.

SIMULATED MARTIAN ENVIRONMENT EXPERIMENTS

Since the last H-SU reports, the Martian environmental apparatus has been redesigned and now offers a flexible system readily adaptable to new data on the Martian environment. It also provides a system with considerably more reliability and ease of handling samples. The apparatus, as designed in Figure 2, has recently been modified again by the addition of another environmental cabinet to be used for conditioning samples to the desired atmosphere. The connections used are almost totally copper and brass, with some glass being used also. The legend of Figure 2 lists the major pieces of this apparatus, with the desired gas mixture being introduced into the right side of the cabinet, and the desired vacuum being regulated with the Cartesian manostat. The chamber is sterilized and dried before use, and all gases are sterilized and dried before they are introduced into the chamber. The chamber and the manifold are subjected to the desired gas mixture, and the vacuum within the manifold is regulated. The atmosphere presently being used is as follows:

Atmosphere	-	99% CO_2 + 1% O_2
Pressure	-	5-7 mb
Moisture	-	Variable (Approximately 100 ppm to 10%)
Nutrients	-	Variable
Temperature	-	-65°C for 16 hrs, +20°C for 8 hrs - Freeze-thaw cycle

Procedures. The general procedures used in subjecting a series of samples to the artificial environment is described here.

1. The desired dry atmosphere is first established in the sterile environmental cabinet by repeated flushing, with the cabinet being left under a positive pressure.

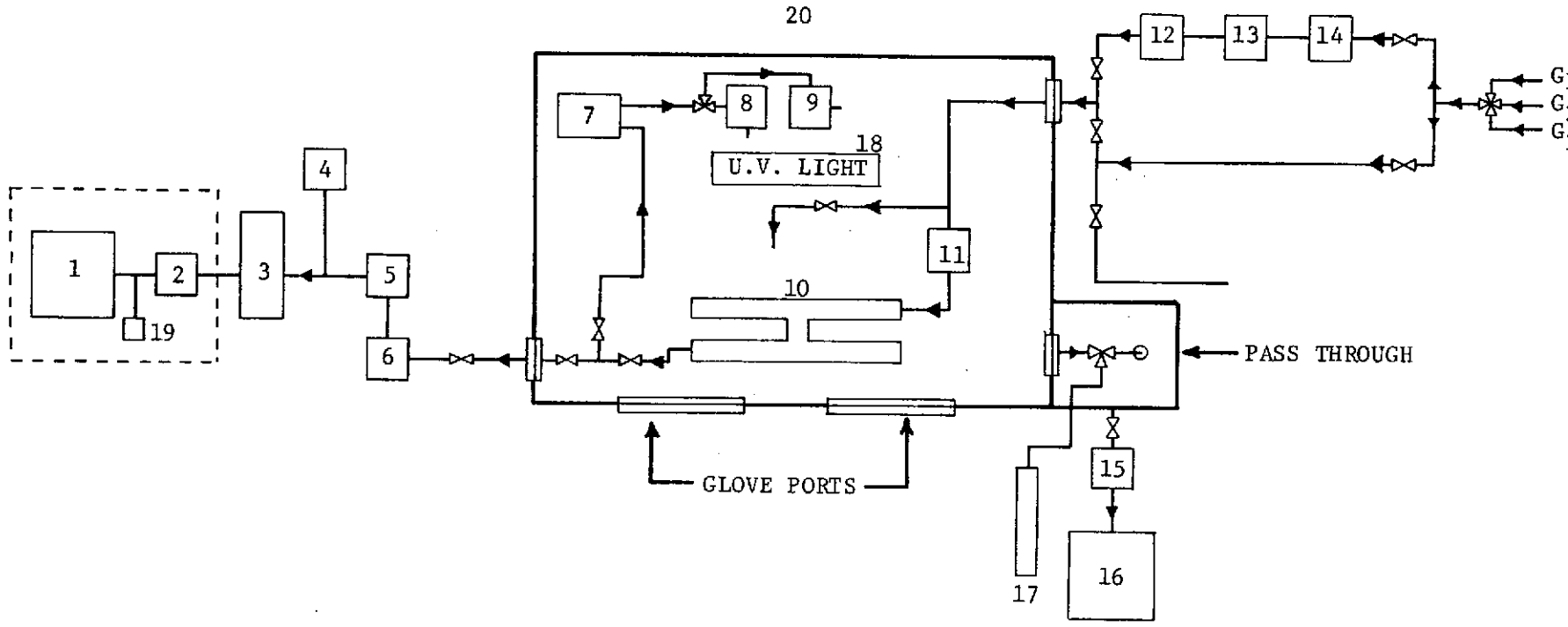


Fig. 2. Block diagram of the apparatus for establishing the experimental Martian environment

1. Vacuum Pump
2. Cold Trap
3. Cartesian Manostat
4. McLeod Gauge
5. Cold Trap
6. Mercury Trap
7. Dyna-Pump
8. Drying Tower
9. Drying Tower
10. 12 Port Brass Manifold with Virtis Vented Adapters
11. Absolute Pressure Gauge
12. Drying Tower
13. Cold Trap
14. Membrane Filter
15. Drying Tube
16. Vacuum Pump
17. Manometer
18. Ultraviolet Light
19. McLeod Gauge
20. Environmental Cabinet

G. Various gas mixtures

2. The ultraviolet light is turned on and left on for 24 hours.

3. Samples to be run are aseptically weighed out into sterile 10 ml. Virtis Vac Vials, and rubber stoppers and plastic caps are applied only loosely.

4. Samples are placed into the cabinet via the access box after the access box has been flushed and placed under the same atmosphere as the entire cabinet.

5. The prepared samples remain in this state of equilibration for various time periods depending on conditions being used. Samples to be dried remain for three days with an exchange of dry gas twice daily, and the gas in the chamber is continuously circulated through drying towers containing activated alumina. Samples to which moisture is added remain for 24 hours in a chamber which contains an atmosphere in which the gas is not dried.

6. Vials containing the equilibrated samples are attached to the Virtis Vented Adapters of the 12-port manifold, and flushed three more times with the gas mixture. A final vacuum of 5 mm Hg is applied to the vials, and they are immediately sealed.

7. The vials, sealed under the proper conditions, are then removed from the cabinet and double-sealed with Virtis Plastic Sealing Bands.

These procedures give the desired artificial environment in a sealed vial which can then be placed into the proper temperature cycle to approximate the Martian environment. Replicate vials are removed periodically and counts performed to study the population dynamics of the sample. This system is also used for the slide-culture technique

which is described later in this report.

Thus far, two runs of the VAB soil through this system have been completed. The samples are incubated at the various temperatures for 21 days with duplicate vials being removed on days 0, 1, 2, 7, 14, and 21, diluted, and plated for incubation at 7°C. One run was performed in which the soil had been dried as described. The second set was set up in identical fashion as the first except that 0.1 ml. of water was added per gram of soil to give excess moisture.

The treatment groups for the first run were as follows:

Groups	Temperature	No. of Sample Vials
1. Simulated Martian Atmosphere	Freeze-thaw	15
2. Simulated Martian Atmosphere	Constant (-65°C)	10
3. " " "	" (-1°C)	10
4. " " "	" (15°C)	10
5. " " "	" (24°C)	10
6. Ambient Atmosphere	Freeze-thaw	10
7. Ambient Atmosphere	Constant (24°C)	10

The treatment groups for the second run were as follows:

Groups	Temperature	No. of Sample Vials
1 - 7. Same as above.		
8. Ambient Atmosphere	Constant (-65°C)	10
9. " "	" (-1°C)	10
10. " "	" (15°C)	10

Ten sample vials were prepared and treated exactly as the treatment vials, then assayed for N_o determinations. The experimental groups using the constant temperatures and the ambient atmosphere are used for two purposes. Some are designed as controls (e.g. Ambient Atmosphere - Freeze-thaw) while others are included to determine growth at constant low temperatures under both a simulated Martian atmosphere and ambient atmosphere.

Duplicate vials are removed and plated in duplicate so that each population is an average of four plates (except for the simulated atmosphere, freeze-thaw in which triplicate samples were used; thus, each population is an average of six plates). All plates were incubated at 7°C and counted after 10-14 days incubation.

Results. The results are given as a comparison of the wet and dry samples. By comparing the various figures, one can compare the effects of moisture, atmosphere (simulated Martian vs. ambient), temperature, etc. Figures 3 and 4 show the effects of moisture on changes in soil populations of the simulated environment with freeze-thaw incubation, and changes in population of pertinent controls. As can be seen there is little overall activity with an increase of more than one log on day 1 of the dry samples, followed by a decrease, then no major changes throughout the remainder of 21 days. The samples with excess moisture showed a change of one log only after 21 days. Of interest is the fact that the experimental tubes seem to demonstrate more active population changes than the control at ambient pressure and 24°C. By comparing the changes of the dry sample vs. the wet sample under the simulated atmosphere with freeze-thaw incubation, it can be seen that the dry sample is less active than the wet one. This is significant because of the very low predicted moisture concentration of Mars.

Figures 5 and 6 show the changes in population of the VAB soil (wet and dry) subjected to the simulated Martian atmosphere and incubated at one of four constant temperatures. These figures show very little activity in the samples with only one sample (24°C in Fig. 5)

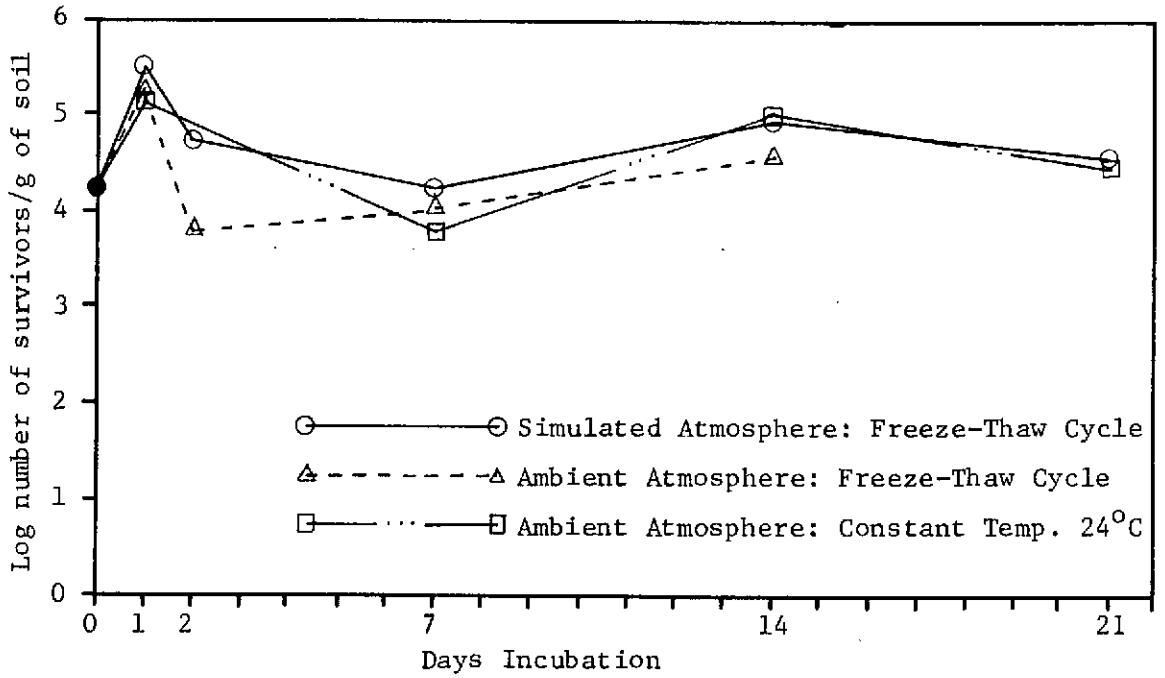


Fig. 3. Response of dry VAB soil to the experimental Martian environment with freeze-thaw incubation

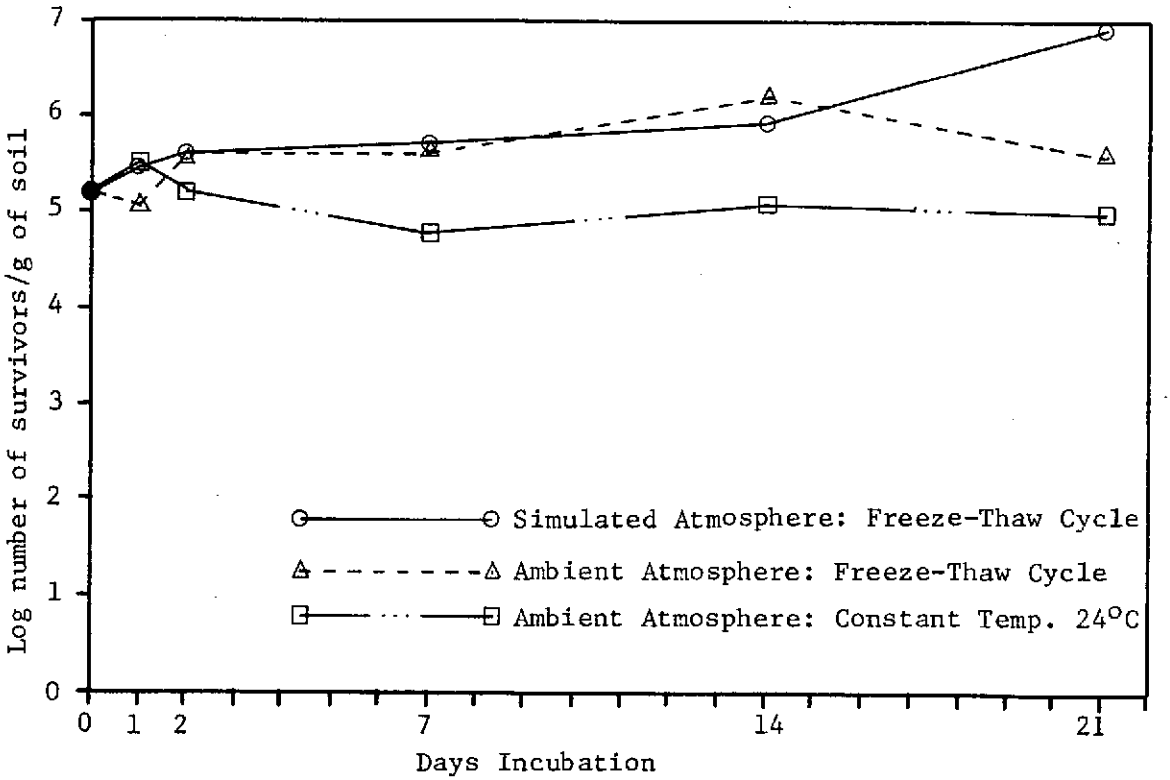


Fig. 4. Response of VAB soil with 10% moisture to the experimental Martian environment with freeze-thaw incubation

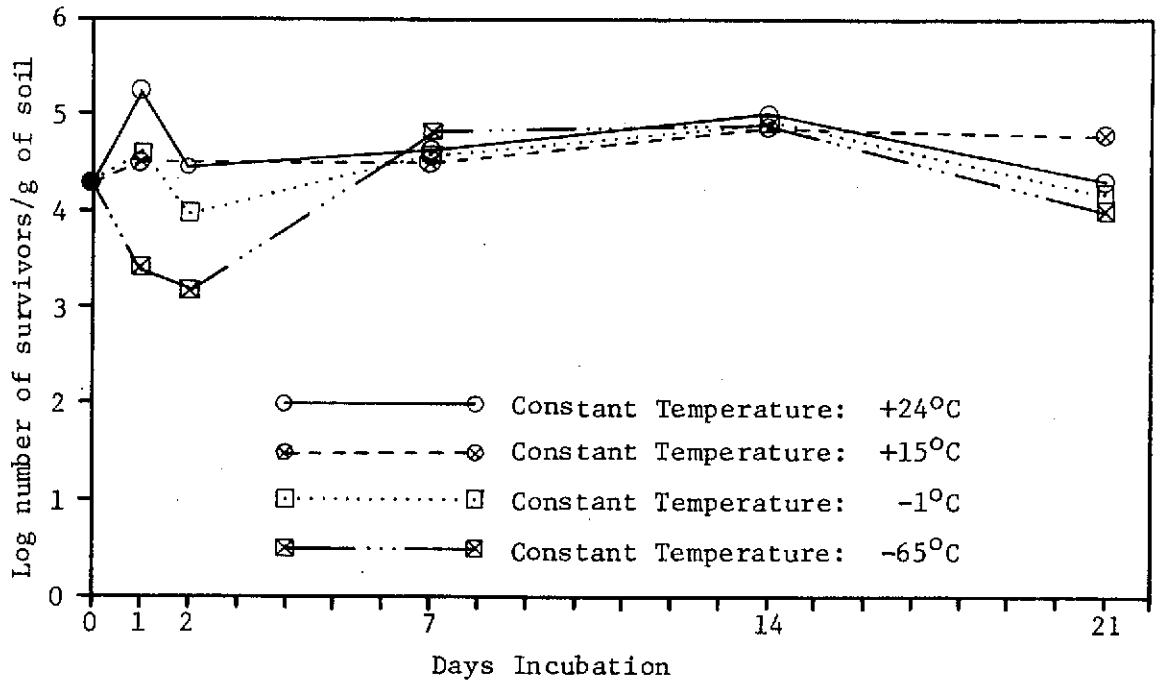


Fig. 5. Response of dry VAB soil to the experimental Martian environment with constant temperature incubation

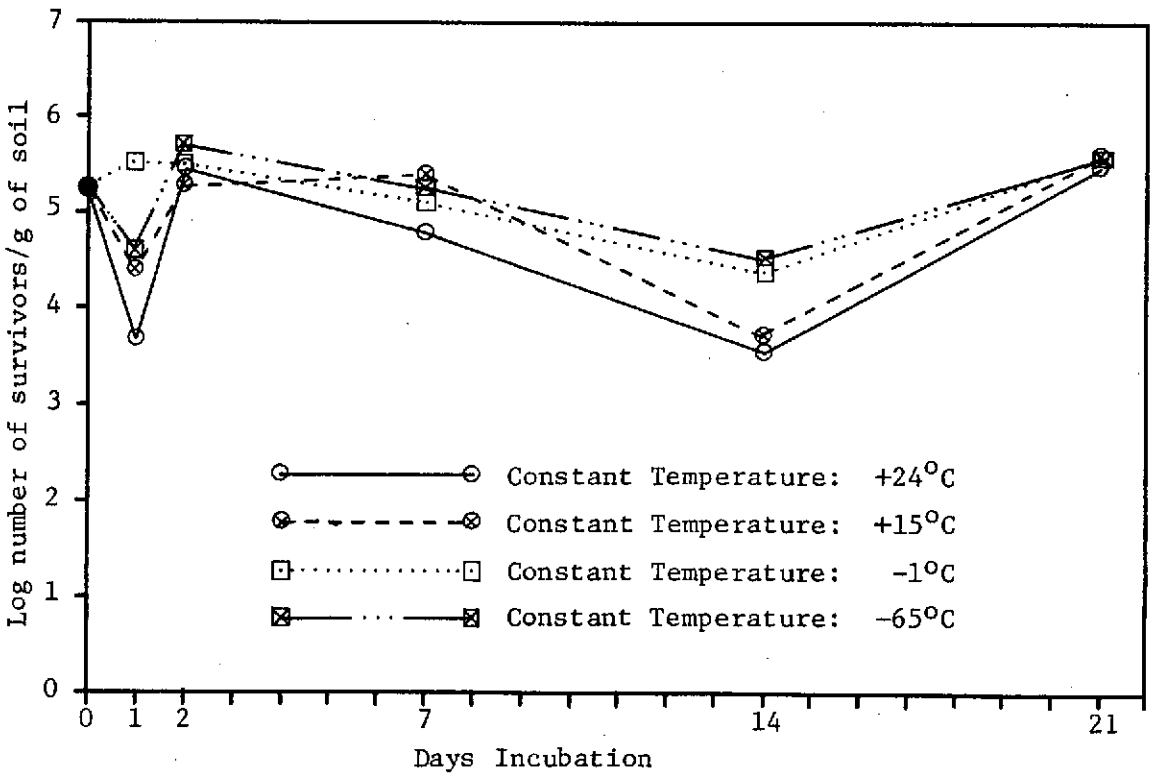


Fig. 6. Response of VAB soil with 10% moisture to the experimental Martian environment with constant temperature

showing an increase of more than one log. This increase occurred on the first day and decreased thereafter.

Figure 7 shows the effect of incubation of the VAB soil under ambient atmosphere at the four constant temperatures. Again there is no appreciable change in populations, and it is of special significance that there was no increase in population of the 24°C sample under ambient atmosphere.

From the foregoing data several observations can be made. First, all samples responded in approximately the same manner under all conditions. Overall there was little or no change in the majority of the populations. Secondly, there is no major decrease in any of the populations. This strongly indicates that the Martian atmosphere used in these investigations is not lethal to the organisms. They appear to remain almost unchanged after 21 days in this environment. Thirdly, the only sample to show any indication of a real increase in population is the sample incubated under the simulated atmosphere and incubated in the freeze-thaw cycle with excess moisture. This treatment seemed to demonstrate a stronger indication of growth than even the ambient atmosphere, 24°C control.

Several samples did show an increase of greater than one log after 24 hrs. incubation, followed by a decrease back to approximately the N_o and very little change throughout the remainder of the 21-day period. In an attempt to explain this, the VAB soil was compared to other Cape Canaveral soils showing similar results from previous investigations (H-SU Report No. 2, July, 1973). By making such comparisons it was seen that the VAB soil and these earlier soils have very similar

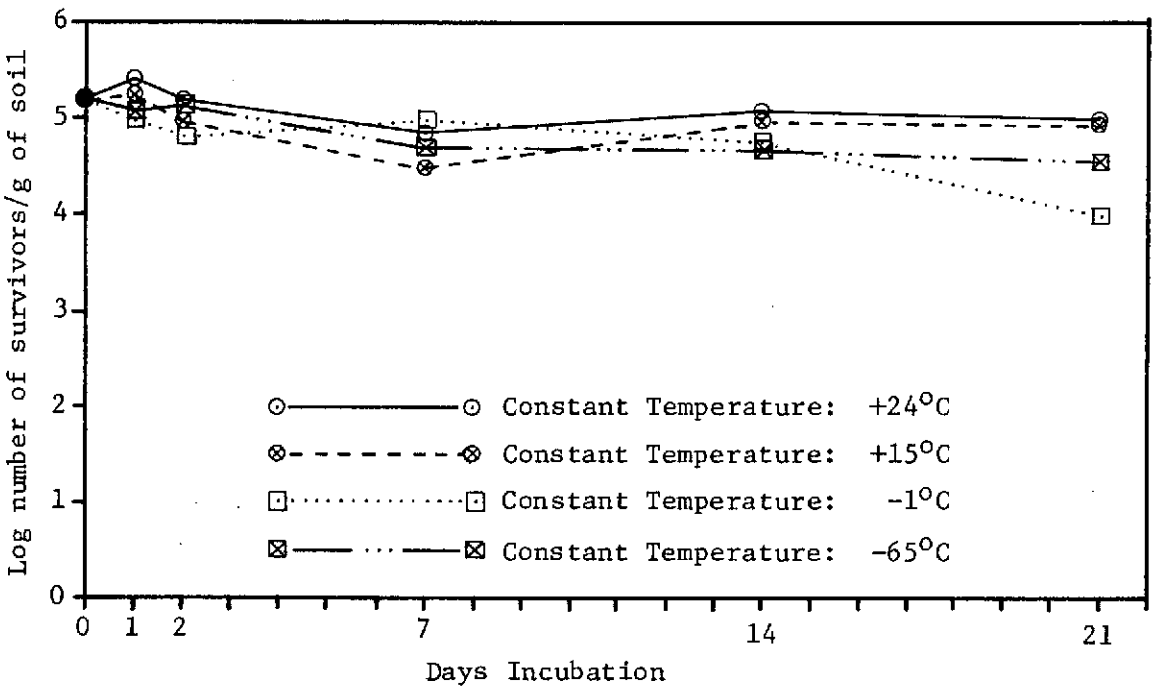


Fig. 7. Response of VAB soil with 10% moisture to ambient atmosphere with constant temperature incubation

composition in that they are primarily sand, with little organic matter. Based upon composition and the pattern of population changes presented in these figures, it is postulated that these soils have very little nutritional value, thus not enabling the organisms to grow. Experiments are now being designed to test this hypothesis,and other Cape Canaveral soils, rich in organic matter, will be run as described here. In addition, numerous other experiments are being designed for testing in this experimental Martian environment. By analyzing the data in the constant temperature experiments in a different perspective, another interesting result can be seen. Figure 8 shows the relationship of incubation of VAB samples at constant temperatures under ambient and under simulated Martian atmospheres. The N_o for each sample is the same and all data points are the average of four plates. As can be seen, the ambient samples respond in approximately the same manner at all four temperatures. This is also true of the samples incubated under the simulated atmosphere. In three of the four samples of the simulated atmosphere there was a decrease of approximately one log on the first day, followed by an increase on the second. This decrease was not seen in the ambient samples, thus it appears that the simulated environment is responsible for the decrease.

The most obvious observation to make in Figure 8 is the consistent decrease in population after 14 days in the simulated environment, followed by an increase in 21 days. If this is real then the organisms begin dying rapidly, but they more than recover within seven days. This pattern of growth appears difficult to accept because of the one log increase in seven days at -65°C. Another possible explanation

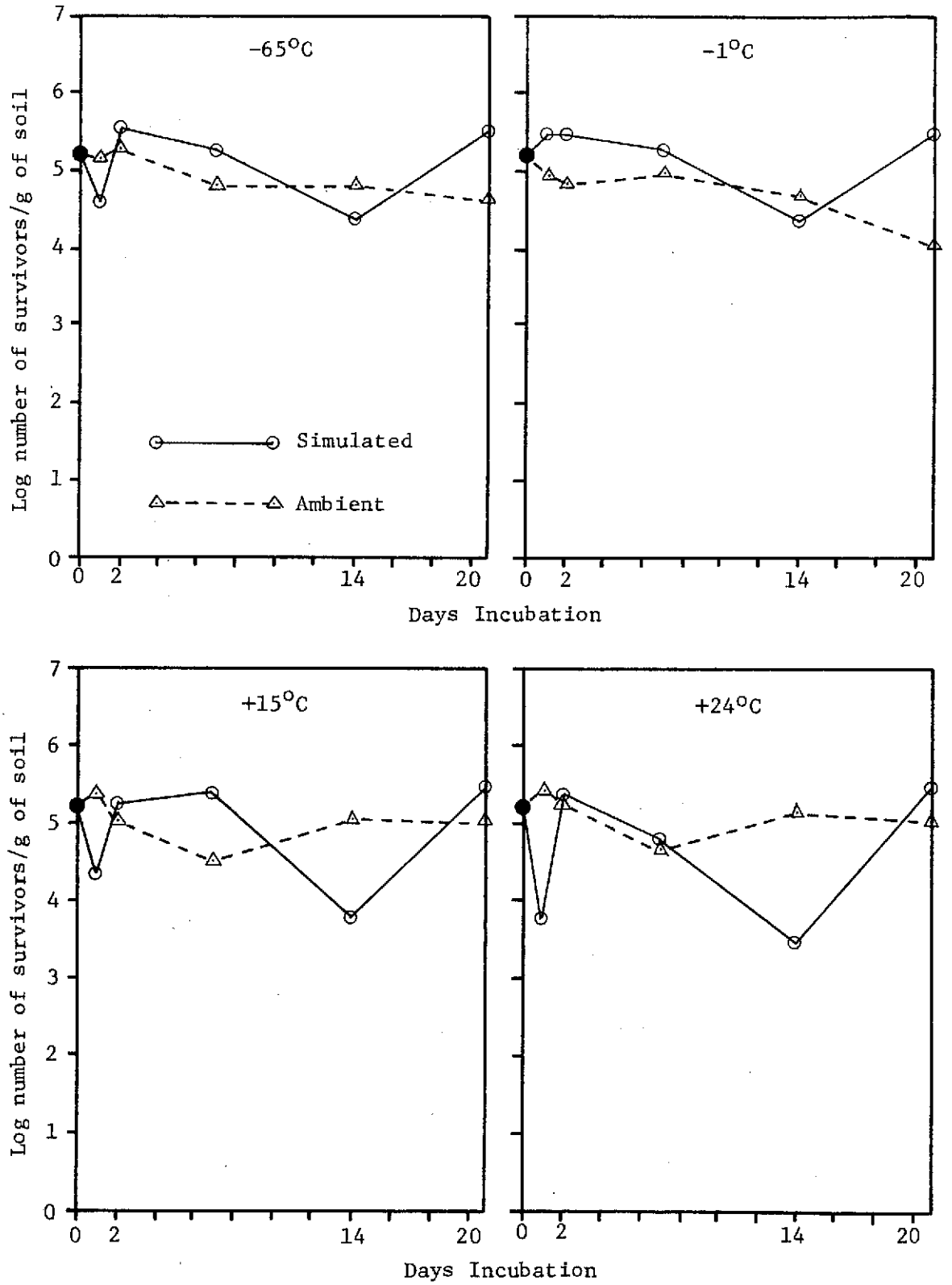


Fig. 8. Response of VAB soil with 10% moisture to the experimental Martian environment and to ambient atmosphere with constant temperature incubation

is that all four samples at day 14 were improperly recorded, and that the data points of these samples are, in fact, one log higher for the subzero samples and two logs higher for the above-zero samples. If this is true, then another obvious result would be that the samples in the simulated atmosphere are consistently higher in population than those of the ambient atmosphere. Regardless of which explanation is the more valid, it appears that the simulated Martian atmosphere is having a favorable effect on soil samples from the VAB.

SLIDE - CULTURE TECHNIQUES

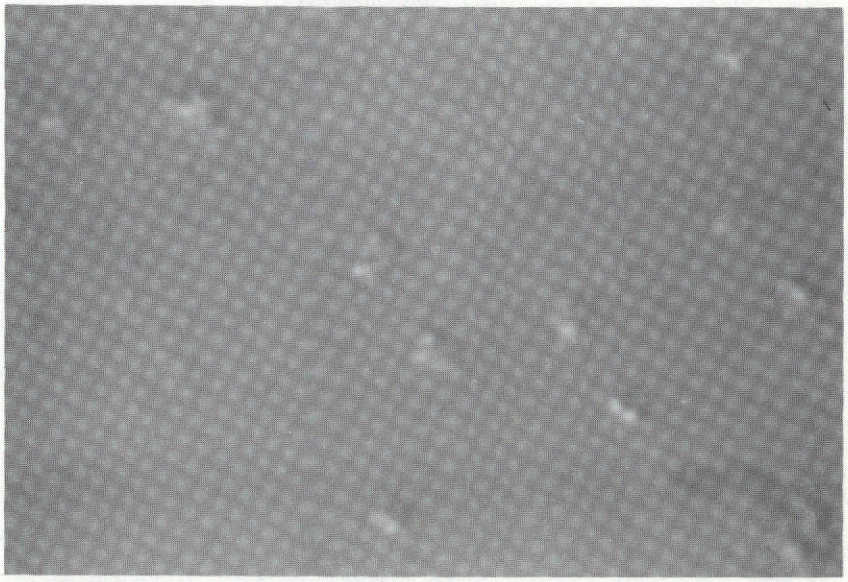
The use of the slide-culture technique was presented in detail in H-SU Reports No. 2 (July, 1973) and 3 (January, 1974). This technique is a modification of that described by A. A. Imshenetsky and is used for rapid detection of growth by the formation of microcolonies on agar-coated slides. Our previous reports described the use of phase-contrast microscopy in these studies, but we have recently gained use of a large Zeiss Universal research microscope which is now equipped with Nomarski differential interference contrast microscopy. As opposed to phase-contrast, which demonstrates internal detail, the Nomarski interference gives a high contrast, high resolution examination of surface structures. It has the additional advantage of showing no light scattering when working with a coated sample (such as our agar-coated slides), and the image is sharply focused on the surface of the agar. These two advantages make the Nomarski interference particularly suited for work dealing with growth of microorganisms on agar-coated slides. This microscope is presently equipped with a 60X dry objective which offers a total magnification range of 750X to 1500X.

Procedures. Detailed procedures for the slide-culture technique were presented in early reports mentioned above. These procedures are presently being modified slightly, and a great deal of our future work will be to standardize procedures for this technique. At present, cultures are inoculated onto various sections of an 8-chambered slide, some chambers containing non-nutrient agar + 0.1% formal-

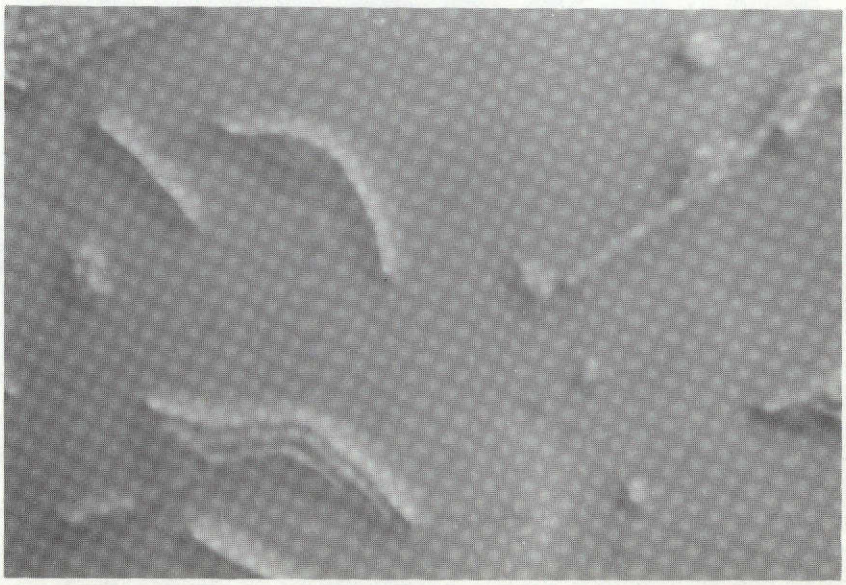
dehyde and others containing a nutrient agar (such as TSA). The slides are prepared and dried prior to inoculation. After inoculation they are then placed into the desired environment and incubated for various periods of time. Slides are removed periodically and examined by Nomarski interference contrast. The non-nutrient agar chamber provides a view of the original inoculum, and this is compared to the TSA chamber. Evidence of growth is demonstrated by the formation of microcolonies in the TSA chamber.

Results. To demonstrate the above procedures, several different types of organisms were inoculated onto the slides and examined hourly over an 8 hour period. Evidence of growth was quite apparent on all samples in 3-4 hours, and Figure 9 demonstrates the type of micrographs obtained. Probably of significance to planetary quarantine is Figure 9A which is a spore suspension of a hardy organism from Cape Canaveral (M6-25) on non-nutrient agar after three hours and Figure 9B, which is the same culture on TSA after three hours. These clearly demonstrate germination and growth in three hours. This technique may have numerous applications in work with spore-formers, and in other areas of planetary quarantine interests.

The micrographs shown in this report are not of the quality expected, but these are some of the very first that have been made with the new microscope, and future micrographs are expected to be of higher quality. Even though they may be of slightly inferior quality at present, they clearly demonstrate rapid detection of growth.



(A)



(B)

Fig. 9. Nomarski interference contrast micrographs (1500X) of spore suspensions of hardy organisms after three hours at 24°C, ambient atmosphere on dry non-nutrient agar (A) and on dry TSA (B)

PREPARATION OF SPORE CROPS OF HARDY ORGANISMS

Cultures of hardy organisms were received from Cape Canaveral in October, 1973. When biochemicals were performed on these, many of them were identified as different organisms from what was reported (H-SU Report No. 3, January, 1974). Subsequently, new cultures were received from Cape Canaveral in Feb., 1974. In the interim, the sample site of the teflon ribbon experiment had been moved from the MSOB to the VAB; therefore, new cultures from the VAB were also received. These new cultures are as follows:

V1-4*	V2-20	V3-13	V5-8
V1-6	V2-32*	V3-28	V5-24*
V1-9	V3-4*	V4-24*	

*Could not subculture

Procedures. As soon as these organisms were received in our lab, the colony morphology and the gram and spore stain characteristics were recorded. They were subcultured onto enriched TSA slants and CTA and lyophilized by washing the fresh TSA slants with 2.0 ml. of 10% skim milk. Biochemical characterization was then performed in duplicate for identification.

Attempts were made, and some are still in progress, to prepare spore crops from these cultures. This was done by preparing 72 hr. broth cultures of the organisms and using this to seed AK-2 Sporulation Agar (BBL) to which was added 0.8 ml of 10% $CaCl_2$ per liter. As the cultures grew, they were examined for spore production, subcultured onto other AK-2 agar plates, and again examined until sporulation occurred.

When a stain showed approximately 80% sporulation, the plates were washed with sterile buffer and the suspension placed into a test-tube for heat-shocking at 80°C for 20 min. After heat-shock the cultures were washed three times with sterile buffer and three times with 95% ethanol and finally stored in 95% ethanol. The suspensions were then titered. After determining the titers of the spore suspensions, they were again examined biochemically (in duplicate) and microscopically to demonstrate that no contamination had occurred in preparation of the spore suspensions.

Results. Of the 32 cultures on which biochemicals were run, four showed differences of three biochemicals, and only two (M2-18 and M5-19) showed differences of more than three biochemicals. Because of the variability of these six organisms, they will not be used in further testing of the hardy organisms.

Upon titering the spore suspensions, the following results were observed:

1	had	a	titer	of	10^2	spores/ml
4	"	"	"	"	10^3	"
3	"	"	"	"	10^4	"
3	"	"	"	"	10^5	"
1	"	"	"	"	10^6	"
5	"	"	"	"	10^7	"
1	"	"	"	"	10^8	"
3	"	"	"	"	10^9	"
1	"	"	"	"	10^{10}	"

Many of these had to be run several times before viable spore crops could be prepared. Results of biochemical testing of these spore suspensions are not complete at the time of this report, but early indications are that the spore suspensions are pure cultures.

COUNTS OF SOIL SAMPLES FROM THE MANUFACTURE AREA OF THE VIKING SPACECRAFT

New soil samples from the manufacture area of the Viking spacecraft were received by our lab, and psychrophilic counts were performed on these according to the methods given in H-SU Report No. 1 (Jan., 1973). Counts were made in duplicate for heat-shocked and non-heat-shocked samples, and plates were incubated at 7°C for 10-14 days. At the time of writing this report, temperature studies had not been completed; therefore, results of the percentage of obligately psychrophilic sporeformers from these samples will be presented in the next report.

The results of the psychrophilic (7°C) counts from these samples are as follows:

M-1	Heat-Shocked	4.8×10^4 CFU/g of soil
M-1	Non-Heat-Shocked	4.7×10^6 CFU/g of soil
M-2	Heat-Shocked	1.3×10^4 CFU/g of soil
M-2	Non-Heat-Shocked	8.3×10^5 CFU/g of soil

These counts are in the same general population range of psychrophilic counts of previous samples from this area, and more complete results will be presented in the next report.

EFFECT OF HEAT SHOCK FOR DIFFERENT TIME PERIODS

Procedure. Because heat-shocking is a routine procedure performed in work with spore-formers, this experiment was designed to determine at what time the non-sporeformers are destroyed in the sample. Because we use dry and wet soil, the experiment is designed to test both conditions. Small (10 ml) vials were used, and 1 gm. of soil from the VAB at Cape Canaveral was placed into these. One set was left dry, and 9.0 ml. of 1% peptone was added to the other set. These were placed in a water bath at 80°C (temperature recorded on 2 thermometers). Two vials, one dry and one wet, had thermometers in them also, to determine how long it took the samples to reach temperature. After reaching 80°C, the timer was started and samples were removed at 10, 15, 20, 25, and 30 minutes and placed immediately into cold water. They were then diluted and plated using the spread plate technique. This technique was used instead of the pour plate so that we could more easily examine the colonies to determine at which time the culture contained only sporeformers. TSA was used as the plating medium.

Three samples dry and three samples wet were used. Their original populations (N_o) were determined just prior to heat-shock. Replicates of the three wet and three dry samples were placed into the water bath, timed after they had reached temperature, and vials of each sample were removed at 5 min. intervals for 10-30 minutes. After being immediately cooled, the samples were diluted in 1% peptone and plated in duplicate for incubation at 7°C and 24°C. The 7°C temperature was used to determine the effects of heat shock on psychrophilic populations. Running three samples

(wet and dry) and plating in duplicate at the two incubation temperatures then gives counts which will be the average of six plates for any one population.

After incubation, the plates were counted and representative plates from each time interval were examined to determine the percent sporeformers on each plate. This was done by examining representative colonies from each plate microscopically and by colony morphology to determine what percent of the total population were sporeformers.

Results. It was observed that the dry soil sample heated only slightly faster than the wet sample. The dry required approximately 6 min. 30 sec. to reach temperature while the soil with 9 ml. peptone required 7 min. 25 sec.

The colonies on the plates of the N_o determinations showed a much more diverse population than any of the heat-shocked samples, and it was observed that there were morphologically different sporeformers on the heat-shocked plates than on those which were not heat-shocked. At 7°C several colonies of molds appeared on the N_o plates, were greatly reduced after 10 min. at 80°C, and were entirely absent from 15-30 min. of heat-shocking.

Counts for the different treatment periods at both 7°C and 24°C are presented in Table 3. As expected, the lower incubation temperature results in a smaller population. The interesting result to note is that the counts do not change appreciably upon longer exposure to the 80°C temperature. It appears that heat-shock is causing little or no change in the population. However, upon examination of plates to determine the percentage of sporeformers, it can be seen that the

Table 3. Counts of VAB soil samples at 7°C and 24°C after heat-shocking for various periods of time[a]

Duration of Heat-Shock	SAMPLE			
	Dry Soil		Dry Soil + 9.0 ml 1% peptone	
	7°C	24°C	7°C	24°C
N_o[b]	8.3×10^4	1.1×10^6	9.1×10^4	2.2×10^6
10 min.	8.3×10^4	4.3×10^5	2.8×10^4	4.3×10^5
15 min.	1.5×10^5	2.9×10^5	6.2×10^4	2.4×10^5
20 min.	3.0×10^4	3.1×10^5	8.4×10^4	4.4×10^5
25 min.	3.9×10^4	3.6×10^5	8.0×10^4	5.0×10^5
30 min.	2.4×10^4	5.8×10^5	1.2×10^5	4.6×10^5

[a]Counts are average of 6 plates given as CFU/g of soil
[b]Original counts

Table 4. Percent of sporeformers in VAB soil at 7°C and 24°C after heat-shocking for various periods of time[a]

Duration of Heat-Shock	7°C	24°C
N_o	16.3%	28.8%
10	83.0%	62.7%
15	89.6%	98.7%
20	93.7%	ND[b]
25	98.0%	79.4%
30	100.0%	100.0%

[a]Average of examination of 6 plates
[b]Not done because of spreaders on the plates

population is indeed changing (Table 4).

After only 10 min. of heat-shocking it appears that many non-sporeforming organisms have been killed, and more are killed as the duration of heat-shocking is increased. After 25 min. of heat-shock and incubation at 24°C, a population of several colonies of branching, Actinomycetes-like organisms was found. They were not present after 15 min. or 30 min., and their presence is as yet unexplained.

By observing Tables 3 and 4 , it can be seen that the soil population appears to be changing in content, but not necessarily in numbers. From the results of these tables, it appears that increasing the duration of heat-shocking induces some of the dormant spores to germinate while destroying some of the non-sporeforming organisms. This concept is substantiated somewhat by the observation of very rough, volcanic-type colonies after prolonged heat-shock which did not appear after 15 min. of heat-shock. As seen in Table 3,it also appears that heat-shocking has basically the same effect on wet and dry soil samples, but that it does take slightly longer for the wet sample to reach temperature. It also appears that heat-shock has basically the same effect on organisms incubated at 7°C or 24°C.

EFFECT OF SPREAD PLATE VS. POUR PLATE ON PSYCHROPHILIC COUNTS

Procedures. It has been reported that the addition of molten agar to a sample using the pour-plate technique has a lethal effect on psychrophilic populations. For this reason, our lab has used the spread-plate technique almost exclusively. Recent experiments have been better suited for the pour-plate technique; therefore, an experiment was designed to compare the effects of these two techniques on psychrophilic populations.

Four replicate samples (1 gm) of soil from the manufacture area (M) of the Viking spacecraft and four replicate samples (1 gm) of soil from Cape Canaveral (C) were aseptically placed into vials and diluted with 1% peptone. These were then plated in duplicate using both the spread-plate and pour-plate techniques and incubated at $7^{\circ}C$.

Results. Final counts are given in Table 5. Analysis of available data by the t test shows no significant difference between use of the pour plate vs. the spread plate at the 0.05 probability level. In almost all cases, the pour plate did yield a somewhat lower population. From these results it can be concluded that the pour plate may indeed be lethal to a small, but apparently insignificant part of the psychrophilic population. More intensive investigation would be necessary to determine the characteristics of this small, susceptible population. Based upon these results then, our lab will use the pour-plate technique for routine population studies and the spread-plate technique when trying to assay a sample for psychrophilic population profiles, or when attempting to isolate obligate psychrophiles.

Table 5. Psychrophilic counts[a] of soil samples from the manufacture area (M) and assembly area (C) of the Viking spacecraft using both the spread-plate and pour-plate techniques

Soil Sample	Spread Plate[b]	Pour Plate
M-I-A	4.4×10^6	1.8×10^6
M-I-B	2.4×10^6	1.9×10^6
M-II-A	4.2×10^7	8.0×10^6
M-II-B	1.4×10^7	7.9×10^6
CA	1.0×10^5	2.0×10^5
CB	1.4×10^5	4.4×10^5
CC	2.7×10^5	9.4×10^4
CG	1.6×10^5	8.9×10^4
M - Average[c]	1.6×10^7	4.9×10^6
C - Average	1.7×10^5	2.1×10^5

[a]Counts are results of duplicate plates
[b]CFU/g of soil
[c]Average of 8 plates